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Characterization of the Voltammetric Response
At Intracellular Carbon Ring Electrodes

by

Yau Yi Lau, Jennifer B. Chien, Danny K. Y. Wong,
Andrew G. Ewing

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Department of Chemistry
Penn State University
University Park, PA 16802

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13. ABSTRACT (Maximum 200 words) → The Intracellular response of ultrasmall carbon ring electrodes has been investigated. Adsorption of high molecular weight species (e.g. proteins, lipids, sugars, etc.) on the electrode surface appears to result in deterioration of the amperometric signal, making <u>in vivo</u> voltammetric measurements difficult. Assuming a linear dependence between the degree of electrode fouling and the number of scans taken in the neuronal microenvironment, an analytical method based on both pre- and post-calibration data is presented to evaluate the response obtained at deteriorating ultrasmall carbon ring electrodes during intracellular voltammetry. Additionally, poly(ester sulfonic acid)-coated carbon ring electrodes have been used in an attempt to increase selectivity in intracellular voltammetry, and to reduce the effects of electrode fouling. <u>In vitro</u> results obtained in solutions of dopamine, dihydroxyphenylacetic acid and ascorbic acid indicate some exclusion of anionic species from the electrode by the negative charged polymer film. Moreover, use of polymer-coated electrodes <u>in vivo</u> results in a decrease in the percentage of electrode fouling to where the degradation of the response is only 45%. This leads to a more reproducible response and better detection limits compared to that of naked electrodes. <i>Key words:</i>			
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CHARACTERIZATION OF THE VOLTAMMETRIC RESPONSE
AT INTRACELLULAR CARBON RING ELECTRODES

Yau Yi Lau*, Jennifer B. Chien[†], Danny K.Y. Wong[‡] and Andrew G. Ewing[§]

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*Ms. Yau Yi Lau: Department of Chemistry, 152 Davey Laboratory, Penn State University, University Park, PA 16802.

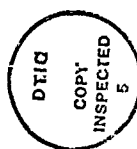
[†]Dr. Jennifer B. Chien, present address: Glaxo Incorporated, 5 Moore Drive, Research Triangle Park, N.C. 27709.

[‡]Dr. Danny K.Y. Wong: Department of Chemistry, 152 Davey Laboratory, Penn State University, University Park, PA 16802.

[§]Associate Professor Andrew G. Ewing: Department of Chemistry, 152 Davey Laboratory, Penn State University, University Park, PA 16802. To whom correspondence should be addressed.

ABSTRACT

The intracellular response of ultrasmall carbon ring electrodes has been investigated. Adsorption of high molecular weight species (e.g. proteins, lipids, sugars, etc.) on the electrode surface appears to result in deterioration of the amperometric signal, making *in vivo* voltammetric measurements difficult. Assuming a linear dependence between the degree of electrode fouling and the number of scans taken in the neuronal microenvironment, an analytical method based on both pre- and post-calibration data is presented to evaluate the response obtained at deteriorating ultrasmall carbon ring electrodes during intracellular voltammetry. Additionally, poly(ester sulfonic acid)-coated carbon ring electrodes have been used in an attempt to increase selectivity in intracellular voltammetry, and to reduce the effects of electrode fouling. *In vitro* results obtained in solutions of dopamine, dihydroxyphenylacetic acid and ascorbic acid indicate some exclusion of anionic species from the electrode by the negatively charged polymer film. Moreover, use of polymer-coated electrodes *in vivo* results in a decrease in the percentage of electrode fouling to where the degradation of the response is only 45%. This leads to a more reproducible response and better detection limits compared to that of naked electrodes.



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INTRODUCTION

There has been a great deal of discussion about the activity of neurotransmitters at the terminal fiber region of a neuron [1-3]. However, there are indications that neurotransmitter activity also takes place in the cell body [4-8]. Using radio-labelling methods, uptake, metabolism, and release by the cell bodies of denervated cholinergic neurons has been observed [5,6]. Furthermore, stimulated release of dopamine has been observed to occur in the substantia nigra *in vivo* [7,8]. An understanding of cellular neurochemistry and cell body involvement in neurotransmission and neuromodulation should be greatly facilitated by measurements at the level of a single cell body. Voltammetric probes used for intracellular voltammetry need to be extremely small. Electrodes having total structural tip diameter smaller than 5 μm have been reported [9-19] and structurally small electrodes have been used to carry out dynamic measurements of neurotransmitters in single nerve cell cytoplasm [9-19]. Carbon ring electrodes have been implanted into a large dopamine neuron of the pond snail *Planorbis corneus* and the voltammetric results obtained suggest that at least 98% of total stores of endogenous dopamine in *Planorbis corneus* are bound and not directly accessible to the cytoplasm [12,13].

Voltammetry can provide a reliable and sensitive technique for detection of electroactive neurotransmitters *in vivo*. However, voltammetric signals tend to deteriorate within minutes after implantation into biological samples [20,21]. This appears to be caused by adsorption of high molecular weight species (e.g. proteins, lipids, sugars, etc.) on the electrode surface. A number of research groups have investigated the use of Nafion-coated micro-electrodes to exclude anionic interferences and also to improve sensitivity at the electrodes

in vivo [22-27]. Electropolymerization of 1,2-diaminobenzene has been reported to prevent interferences and fouling of electrochemical biosensors [28]. Wang *et al.* have explored the application of poly(ester sulfonic acid) on conventional-sized and micro carbon electrodes [29-31]. Poly(ester sulfonic acid) adheres strongly to the carbon surface and the assembly appears to exhibit encouraging permselective, cation-exchange and antifouling properties. In parallel work aimed at improving cation-exchange characteristics of electrodes, Baur [32] has recently reported the electropolymerization of a quaternized poly(4-vinylpyridine)/vinyl ferrocene copolymer onto carbon electrodes as an anion-selective sensor for neurotransmitters.

In this communication, the intracellular voltammetric response of ultrasmall carbon ring electrodes placed in single cell cytoplasm is examined. Loss of electrode response during the experiment has prompted us to employ a procedure involving both pre- and post-calibrations to characterize electrodes used in intracellular voltammetry. Additionally, in this work, carbon ring electrodes have been coated with poly(ester sulfonic acid) in an attempt to enhance voltammetric selectivity between neurotransmitters and to minimize deterioration of electrode performance *in vivo*.

EXPERIMENTAL

Chemicals

In vitro work was performed in a pH 7.4 snail Ringer's solution [33] (39 mM NaCl, 1.3 mM KCl, 4.5 mM CaCl₂, 1.5 mM MgCl₂, 6.9 mM NaHCO₃) or in a pH 7.4 citrate/phosphate buffer prepared with doubly distilled water. Dopamine (DA), ascorbic acid (AA), and dihydroxyphenylacetic acid (DOPAC) (Sigma Chemical Co.) were used as received. All catechol

solutions were deoxygenated (N_2) for 20 min prior to experiments and a blanket of nitrogen was then maintained over the solution. Eastman-AQ55D poly(ester sulfonic acid) (28% dispersion), obtained from Eastman Kodak Company, was used as received.

Electrodes and Apparatus

Ultrasmall carbon ring electrodes were constructed as described previously [10,19]. Carbon ring electrodes were voltammetrically tested with DA in citrate/phosphate buffer (pH 7.4), as described previously [10,19,35]. Only electrodes which showed well-defined sigmoidal cyclic voltammograms for oxidation of DA, and good sensitivity (i.e. detecting $<5 \mu M$ DA), were used in subsequent experiments. Polymer-coated carbon ring electrodes were obtained by dipping electrodes in a poly(ester sulfonic acid) solution [1:20 polymer/acetone (v/v)] 10 times (1 second each), and then air drying for approximately 15 min.

Cyclic voltammetry was carried out with a locally constructed low-current three-electrode potentiostat [21]. All electrochemical measurements were carried out in a copper mesh Faraday cage. *In vitro* experiments were performed in a 30 mL glass vial with three holes drilled in a plastic cap to accommodate the three electrode system. A sodium saturated calomel electrode (SSCE) served as the reference electrode and a platinum wire as the auxiliary electrode. Waveslopes of all cyclic voltammograms were calculated from a plot of $\log \frac{I_\infty - I}{I}$ (where I denotes current and I_∞ the limiting current on the voltammogram) vs potential, and the half-wave potential ($E_{1/2}$) being the intercept on the potential axis.

***In vivo* Preparation**

Planorbis corneus were obtained from NASCO (Fort Atkinson, WI) and were maintained in aquaria at room temperature until used. The snails were pinned in a wax-filled petri dish and dissected under snail Ringer's solution to reveal the left and right pedal ganglia. A micromanipulator (de Fonbrune, Curtin Matheson) was used to place electrode into the identified dopamine neuron [34]. The electrode potential was monitored (voltage follower and oscilloscope) vs a platinum wire during implantation. A negative shift in potential was indicative of cell penetration. Following implantation, the carbon ring working electrode was connected to the potentiostat. The SSCE reference electrode was placed via a salt bridge into the Ringer's solution with a platinum wire serving as the auxiliary electrode.

Local Bathings

Local bathing of the outside of the neuron with dopamine was achieved by pressure injection through a glass micropipet. The injector consisted of standard capillary glass (6270 A-M Systems) pulled down to small tip diameters with a microelectrode puller (Harvard Instruments) and cleaved with a scalpel to approximately 100 μm tip diameter. The tip of the injector was then manipulated (Prior manipulator, Medical Systems) to a distance of approximately 100 μm from the outside of the cell body.

Intracellular Measurements

Staircase voltammograms (-0.2 to 0.8 V, 50 steps per linear scan, 20 mV/step and 100 ms/step (i.e. 0.2 V/s), 2 s delay between scans) were collected using a locally constructed potentiostat, an IBM personal computer, and locally written software. The experimental

protocol involved repeated voltammograms in series of 100 scans. Plots of oxidation current vs time were generated by use of one potential step in each voltammogram.

RESULTS AND DISCUSSION

Calibration of the Intracellular Voltammetric Response

Intracellular voltammetry is carried out by implantation of an electrode into the cell body of the DA neuron of *Planorbis corneus*. For the experiments reported here, the cell is bathed with a 0.5 mM DA solution. DA entering the cell is monitored intracellularly with repeated voltammetry in which the potential is scanned between -0.2 and $+0.8$ V. Figure 1 shows the oxidation current monitored at the $+0.78$ V of repeated voltammograms obtained intracellularly following repeated bathing of the cell with 0.5 mM DA. The rising portion of the oxidation current in each peak appears to represent active transport of DA across the cell membrane. The rapid decay in monitored oxidation current following DA increase appears to represent DA metabolism and/or vesicularization in this cell. The peak current response provides a measure of the amount of DA transported into the cytoplasm from outside the cell. The peak current for repeated bathing of the cell with DA decreases gradually as a function of time. This appears to be the result of a loss of electrode sensitivity rather than a physiological process.

Figure 1

Loss of voltammetric response is apparently due to specific adsorption of biological species (e.g. proteins, lipids, sugars, etc.) on the electrode surface. These adsorbed species

inhibit analyte molecules from diffusing to the electrode surface and thus make it difficult to carry out quantitative analysis. We have examined methods involving electrode calibration before (pre) and after (post) the *in vivo* experiment. In each case, electrodes were calibrated using solutions of increasing (by standard addition) DA concentration (typically from 5 μM to 100 μM) in citrate/phosphate buffer. This technique allows the analysis of successive current vs time plots. However, it should be emphasized that this procedure is an approximation. For each experiment the extent and progression of electrode fouling is unpredictable and may follow a non-linear path. Figure 2 shows *in vitro* pre- and post-calibration plots for a carbon ring electrode used to obtain 1000 voltammograms in the intracellular environment. A decrease in electrode sensitivity for DA is observed after voltammetry is carried out in the cytoplasm of the giant dopamine cell.

Figure 2

For quantitative measurements of analyte concentration *in vivo*, it has been suggested that post-calibrations should be used for the best representation of an electrode's *in vivo* response [21]. While quantitating *in vivo* responses by pre-calibration curves assumes no degradation of the sensor occurs, use of post-calibration curves assumes essentially all of the decrease in electrode response occurs before any data is collected or in the first few scans. For the repeated DA bathing studies reported here, we propose that the loss in electrode response is better compensated for by assuming the electrode degradation occurs in a linear fashion throughout the *in vivo* experiment. This situation is shown schematically in Figure 3. The "linear-average calibration" method assumes that the degree of fouling of the electrode surface is linearly dependent on the number of scans taken in the neuronal microenvironment.

In this method, the value for the concentration observed intracellularly is based upon both the plots in Figure 2.

Figure 3

Quantitative information is obtained with the linear-average calibration method as follows. Staircase voltammograms are carried out intracellularly in sets of 100. Each successive set is plotted on the x-axis as a pseudo time axis. On this axis the sensitivity of the electrode is plotted for the pre-calibration and post-calibration at times 0 and $m+1$, respectively. Here, m is the number of 100 voltammogram sets carried out intracellularly. The calibration line generated by these two points (Figure 3) is then used to determine the intracellular DA concentration from the measured peak current in a particular set of voltammograms (m). Figures 4(a) and 4(b) show plots of peak oxidation current vs time for a series of DA bathings with post-calibration and linear-average calibration methods, respectively. The effect of applying the linear-average calibration scheme on the data in Figure 1 gives rise to a peak concentration that is more stable over several applications of DA.

Figure 4

When the linear-average calibration method is used to examine the amperometric signal at the electrode, peak cytoplasmic DA concentrations and clearance rates for repeated applications of extracellular dopamine in individual snails are observed to be fairly reproducible. Data from six sets of experiments are tabulated in Table 1. Since in each case the

data were obtained from repeated bathings of the same nerve cell, the small error obtained in the concentration observed lends support to the use of the linear-average calibration method. This is also supported by the small variance in the concentrations shown as a function of bathing number in Figure 4. The rate of clearance was determined at the point where the rate of decay in the monitored oxidation current following DA application shown in Figure 1 was maximal. While repeated measurements in an individual snail show good agreement, as observed in Table 1, there is a significant variation in results from different animals, as might be expected.

Table 1

Voltammetric Response of Polymer-Coated Carbon Ring Electrodes

A number of voltammetric studies on the use of poly(ester sulfonic acid)-coated electrodes have recently been reported [29,30,31]. Application of this polymer can be carried out by dip-coating the electrode in a dilute solution of polymer in acetone. Strong adherence of the polymer to the electrode substrate has been reported [29,31]. Also, in these studies, the anionic polymer film inhibits the movement of anionic species (e.g. DOPAC) to the electrode surface, whereas cations (e.g. DA) easily diffuse or ion exchange in this medium. This favorable partitioning effect for cations results in a larger current response compared to that obtained at naked macroelectrodes. Furthermore, previous results [30,31] have also shown that electrode fouling in the presence of proteins *in vitro* can be prevented by coating the electrode with the polymer film.

In the present work, we have coated 3–5 μm total diameter carbon ring electrodes with poly(ester sulfonic acid). Figure 5 shows cyclic voltammograms obtained in 1.0×10^{-4} M DA at a 4 μm carbon ring electrode before and after coating. In both experiments, a well-defined sigmoidal response for the oxidation of DA with very little charging current is obtained. A reduced current response is obtained at the polymer-coated carbon ring electrode. This is, however, in agreement with results obtained at polymer-coated graphite-epoxy microelectrodes (130 μm i.d. and 0.5 mm o.d.) [31]. It appears that the layer of polymer film cast on the carbon ring electrode may hinder the diffusion of DA to the electrode surface, resulting in a lower oxidation current. Engstrom *et al.* [36] have shown that at Nafion-coated microelectrodes, significant distortion of events occurring in the subsecond time domain, caused by the polymer film, result in a small increase in response time of electrodes and thus a decrease in sensitivity.

Figure 5

In our work, a relatively thin polymer film has been cast on the carbon ring electrodes by dip-coating in a 1:20 polymer/acetone (v/v) solution. Thicker polymer films (using 1:10 and 1:5 polymer/acetone solutions) have also been investigated but this gives rise to voltammograms with larger charging current and a lower signal-to-noise ratio. Apparently, thicker films more severely hinder mass transport of material to the electrode surface in these experiments. The small structural dimension of the electrodes employed here precludes microscopic examination of the morphology of the assembly.

It is important to discriminate against DOPAC and AA during measurement of DA since all three species are oxidized in a similar potential range, yielding severely overlapping

voltammetric signals. Detection of DA is made even more difficult when DA is present in a much lower concentration than DOPAC and AA. For example, Gonon *et al.* [37] estimated extracellular concentrations of 306 μM for AA, 17.7 μM for DOPAC and less than 50 nM for DA in the striatum of unanesthetized rats. Further, oxidation of DA in the presence of AA follows an EC' mechanism [38,39,40], where the oxidation product of DA (DA-o-quinone) in solution is reduced by AA to DA which can again be oxidized at the electrode surface. As a result, a given concentration of DA often generates a larger oxidation current in the presence of AA than in its absence. Although AA does not appear to be present in the large DA neuron of *Planorbis corneus*, DOPAC is a major concern as an interferent in this system, whereas ascorbic acid is of general interest in this area. The effect of DA oxidation *in vitro* in the presence of DOPAC and AA have been examined here.

The oxidation of 1.0×10^{-4} M DA at poly(ester sulfonic acid)-coated electrodes has been investigated in two separate experiments prior to and after additions of DOPAC or AA to the solution. Both DOPAC and AA exist as anionic species while DA is cationic in pH 7.4 buffer. Voltammetry of DOPAC at carbon ring electrodes is generally irreversible having waveslopes greater than 140 mV/decade [35]. Voltammetry in solutions of DA and AA involves an EC' catalytic reaction which results in an irreversible looking DA voltammogram. Figure 6 and Figure 7 show a series of cyclic voltammograms for DA oxidation at both naked and polymer-coated carbon ring electrodes in solutions of DA and DOPAC or DA and AA, respectively. The effect of the polymer film has been examined qualitatively in these studies, since it is difficult to compare the magnitude of the oxidation current, as it always becomes smaller at polymer-coated electrodes. In general, both the waveslope and $E_{1/2}$ of the voltammograms do not show any significant difference for either naked or polymer-coated

electrodes in solutions containing DA and DOPAC or DA and AA. The waveslope of DA voltammograms obtained with naked electrodes (Figure 6) in solutions containing DOPAC in the concentration range from 2.0×10^{-5} M to 2.9×10^{-4} M changes from 160 mV/decade to 220 mV/decade. Since these voltammograms do not clearly attain a limiting current, these waveslopes are estimates obtained by using the limiting current measured with only DA in solution. The $E_{1/2}$ of these voltammograms changes from 310 mV to 410 mV. When polymer-coated electrodes are used in identical solutions, the corresponding change in waveslope is from 150 mV/decade to 230 mV/decade and the $E_{1/2}$ value changes from 310 mV to 440 mV.

Figure 6

Figure 7

Similarly, for voltammograms with naked electrodes in solutions of DA and AA (Figure 7), when the AA concentration is increased from 2.0×10^{-5} M to 2.9×10^{-4} M, the waveslope changes from 150 mV/decade to 220 mV/decade and $E_{1/2}$ changes from 340 mV to 500 mV. The use of polymer-coated electrodes results in waveslopes changing from 180 mV/decade to 220 mV/decade and $E_{1/2}$ from 350 mV to 460 mV. These results seem to indicate that oxidation of DA is largely diffusion-controlled at both naked and polymer-coated carbon ring electrodes.

The voltammetric response for DA oxidation is sigmoidal at naked electrodes in the presence of up to 5.0×10^{-5} M DOPAC (Figure 6). At higher concentrations of DOPAC (see Figures 6(i)b-d), distortion of the sigmoidal response becomes significant. In contrast, a

comparatively well-defined sigmoidal response is still observed at polymer-coated electrodes in the presence of 1.5×10^{-4} M DOPAC. Similarly, in Figure 7, a well-defined sigmoidal response for the oxidation of DA at naked electrodes is only observed in the presence of up to 1.0×10^{-4} M AA. The sigmoidal voltammogram obtained for DA at polymer-coated electrodes is not significantly affected by the addition of 2.0×10^{-4} M AA. These results show the effective cation-exchange property of the negatively charged poly(ester sulfonic acid) film deposited on carbon ring electrodes. This selective response is associated with the preference of the hydrophobic polyester backbone for hydrophobic cations [31]. Previous reports appear to have indicated a more effective rejection of AA than DOPAC in experiments involving oxidation of AA or DOPAC alone at poly(ester sulfonic acid)-coated graphite-epoxy microelectrodes [31]. Use of ultrasmall electrodes in this work also appears to minimize the catalytic oxidation current for DA in the presence of AA. Hence, *in vitro* results presented in our work suggest that detection of DA should be possible in the presence of a high concentration of either DOPAC or AA without significant interference from these anions.

Response of Polymer-Coated Electrodes *In Vivo*

The response of poly(ester sulfonic acid)-coated carbon ring electrodes has been examined *in vivo*. For these experiments, intracellular voltammetry has been carried out in the DA cell of *Planorbis corneus*. In this case, electrodes were placed in the cytoplasm and 300 voltammograms were obtained. Figures 8(a) and 8(b) compare the pre- and post-calibration plots obtained at a naked and a polymer-coated electrode, respectively. The absolute currents observed vary between the two electrodes due to variability in electrode construction. Figure 9 shows the current sampled at +0.62 V as a function of time for the two electrodes during

intracellular voltammograms in single cell cytoplasm with no stimulation or acquisition of repeated application of DA. At the naked electrode, the current decays rapidly to a base line level after approximately 7 voltammograms are carried out (~ 50 s). In contrast, the corresponding current at the polymer-coated electrode decays over a longer period of time (~ 150 s). However, the change in background oxidation current is smaller for the polymer-coated electrode relative to the naked electrode. The significance of this result is unclear because the data do not appear to correlate to the calibrations, but rather to any initial changes in the chemistry of the environment of the electrode tip. The decay observed could represent a change in the concentration of oxidized species rather than a change in electrode response. It is important to make this distinction, since interpretation of the data as only electrode fouling leads to the hypothesis that post-calibration data should be most useful. This is clearly not suggested by the data presented in Figure 4.

The result of comparison of pre- to post-calibration data for polymer-coated and naked electrodes is significant. Comparison of the current observed at a specific concentration leads to a smaller current on the post-calibration plot relative to that on the pre-calibration plot, for both naked and polymer-coated electrodes. This indicates a certain degree of electrode fouling after 300 voltammograms (~ 30 min) in the cytoplasm. From the calibration plots, the average ratio of two current values from pre- and post-calibration, selected in the lower and higher concentration range, respectively, has been used to calculate the percent electrode response remaining after the intracellular experiment. Additionally, the detection limit has been determined by comparison of values of limiting current to twice the peak-to-peak noise level obtained for currents measured at a single potential for repetitive voltammetry. The average loss of response is $74 \pm 3\%$ (standard error of the mean (SEM); $n=6$) with an

average detection limit of 12 μM when naked carbon ring electrodes (of structural diameters 2 to 8 μm) are used. In comparison, an average loss of response of $45\pm4\%$ (SEM; $n=5$) and an average detection limit of 6 μM are obtained when polymer-coated electrodes (of structural diameters 2 to 7 μm) are used. The mechanism of this resistance to fouling is not clear, but could involve restricted mass transfer of large molecules to the electrode.

Figure 8

Figure 9

In conclusion, an analytical method which assumes a linear dependence of the degree of electrode fouling on the number of scans obtained intracellularly has been proposed in this work. A more realistic quantitative estimation of neurotransmitters present in the dopamine cell of *Planorbis corneus* following DA bathing has been obtained using this method. Additionally, it has been demonstrated that electrode fouling on ultrasmall carbon ring electrodes can be reduced from 74% fouled to 45% fouled by use of a poly(ester sulfonic acid)-coating. Detection limits at these electrodes are also improved by a factor of two.

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FIGURE CAPTIONS

Figure 1 Observed time course plot of repeated dopamine bathings (0.5 mM, 30 μ L each arrow) with the electrode placed in the cell body of the identified dopamine neuron. The dopamine solution used for extracellular bathings was made up in the modified snail saline described in the text. The current was monitored at +0.78 V vs SSCE with 7 s between measurements.

Figure 2 Typical *in vitro* calibration plots obtained for DA at + 0.78 V vs SSCE with a carbon ring electrode. Plots obtained before (pre) and after (post) an intracellular experiment of 1000 voltammograms.

Figure 3 Predicted electrode response to a particular DA concentration as a function of the number of scans taken intracellularly.

o — concentration points obtained by pre- and post-calibration plots.

abscissa: concentration indicated by a measured current level.

ordinate: approximate number of scans taken with the electrode.

Use of pre-calibration data assumes no change in electrode response during the experiment (indicated by -o-). Use of post-calibration data assumes the electrode response changes only during the initial portion of an *in vivo* experiment (- - -). The linear-average calibration method assumes a constant decrease in electrode response during the *in vivo* experiment (—).

Figure 4 Concentration vs time for the repeated DA uptake experiments shown in Figure 1. Each circle (o) represents the peak concentration of DA for the current responses shown in Figure 1. (a): results obtained when post-calibrations are used to determine intracellular concentrations; (b): results obtained when the linear-average calibration method shown in Figure 3 is used.

Figure 5 Cyclic voltammograms for oxidation of 1.0×10^{-4} M DA in citrate/phosphate buffer (pH 7.4) at a $4 \mu\text{m}$ carbon ring electrode (a) before and (b) after being coated with poly(ester sulfonic acid). Scan rate = 100 mV s^{-1} .

Figure 6 Oxidation of 1.0×10^{-4} M DA with varied concentrations of DOPAC present in pH 7.4 citrate/phosphate buffer (pH 7.4) at a $4 \mu\text{m}$ carbon ring electrode (i) before and (ii) after being coated with poly(ester sulfonic acid). DOPAC concentration: (a) 2.0×10^{-5} M, (b) 5.0×10^{-5} M, (c) 1.5×10^{-4} M and (d) 2.9×10^{-4} M. Scan rate = 100 mV s^{-1} .

Figure 7 Oxidation of 1.0×10^{-4} M DA in the present of AA at a $4 \mu\text{m}$ carbon ring electrode (i) before and (ii) after being coated with poly(ester sulfonic acid). AA concentration: (a) 2.0×10^{-5} M, (b) 1.0×10^{-4} M, (c) 2.0×10^{-4} M and (d) 2.9×10^{-4} M. Scan rate = 100 mV s^{-1} .

Figure 8 *In vitro* pre- and post-calibrations obtained in various concentrations of DA at +0.62 V vs SSCE with (a) 8 μm naked, (b) 7 μm poly(ester sulfonic acid)-coated carbon ring electrodes.

Figure 9 Current (sampled at +0.62 V) vs time plot obtained with (a) 8 μm naked and (b) 7 μm poly(ester sulfonic acid)-coated carbon ring electrodes during intracellular voltammetry.

Table 1 Reproducibility of the intracellular voltammetric
response to repeated applications of extracellular DA

Number of bathings	[DA] and volume applied (mM)	Intracellular [DA] ^(a) (μM) mean \pm SEM	Maximum rates of DA clearance ($\mu\text{M s}^{-1}$) mean \pm SEM
7	0.25 (30 μL)	29.9 \pm 2.3	0.873 \pm 0.153
6	0.25 (25 μL)	22.8 \pm 1.5	0.285 \pm 0.013
8	0.25 (20 μL)	58.1 \pm 1.4	0.638 \pm 0.136
5	0.50 (50 μL)	65.3 \pm 2.6	2.65 \pm 0.54
7	0.50 (30 μL)	34.9 \pm 1.1	0.338 \pm 0.018
4	1.0 (25 μL)	44.1 \pm 4.5	0.747 \pm 0.202

Each entry line represents n multiple bathings on a single snail.

SEM — standard error of mean.

^(a)Maximum concentration observed intracellularly after extracellular bathing with DA.

